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14. ABSTRACT
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We hypothesized that dehydroepiandrosterone (DHEA) metabolites or their synthetic derivatives are able to bind to the androgen receptor with low, if any, agonist activity and thus function as better antiandrogens than currently available ones. We previously identified three potential compounds with marginal androgenic activity. Nonetheless, we were aware that these compounds also possessed estrogenic activity that may cause severe side effects if used *in vivo*. Using different prostate cancer cell lines, we evaluated androgenic/antiandrogenic effects of the three steroids on cell proliferation/survival/apoptosis and expression of several molecules related to cell growth/angiogenesis/metastasis. We found that these compounds indeed exhibited antiandrogenic activities, although they were not always significant. Our results suggest that these compounds are superior to current antiandrogens, in terms of androgenic and antiandrogenic properties in prostate cancer cells *in vitro*. We also established LNCaP sublines after long-term treatment with each compound. Additionally, screening of DHEA derivatives resulted in identification of a new compound that shows marginal androgenic/strong antiandrogenic activity as well as marginal estrogenic activity. We continue to assess the effects of these four potential compounds in prostate cancer *in vitro* and *in vivo*.

15. SUBJECT TERMS
Antiandrogen, androgen receptor, dehydroepiandrosterone, androgenic activity, estrogenic activity

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Introduction

Although antiandrogens that can block androgen action through the androgen receptor (AR) have been widely used for the treatment of prostate cancer, the majority of available ones possess agonist activity, resulting in increase in serum prostate-specific antigen (PSA) levels, known as the antiandrogen withdrawal syndrome [1,2]. In addition, we previously found that androstenediol (Adiol), a physiological metabolite from dehydroepiandrosterone (DHEA) and a precursor of testosterone, has androgenic activity which was not completely antagonized by two clinically used antiandrogens, hydroxyflutamide (HF) and bicalutamide (BC) [3]. Therefore, new and more effective antiandrogenic compounds with marginal androgenic activities need to be identified. Our hypothesis was that DHEA metabolites or their synthetic derivatives are able to bind to the AR with low, if any, agonist activity and thus function as better antiandrogens than currently available ones. We previously screened DHEA derivatives/metabolites for their androgenic and antiandrogenic activities and found that three compounds, 3β-acetoxyandrost-1,5-diene-17-ethylene-ketal (ADEK), 3β-hydroxyandrost-5,16-diene (HAD), and 3-oxo-androst-1,4-diene-17-ketal (OADK), show only marginal agonist effects and suppress significantly 5α-dihydrotestosterone (DHT)- and Adiol-induced AR transactivations [4-6]. Thus, ADEK, HAD, and OADK have the potential to function as potent antiandrogens that carry fewer risks of withdrawal response if used for therapy in prostate cancer patients. However, the disadvantage of using these compounds in clinical settings includes their estrogenic activity [5,6] that induces chemical castration and that may also cause severe side effects, such as cardiovascular toxicity. The task in the approved Statement of Work in this period (months 1-12) would be to evaluate the effects of DHEA derivatives *in vitro* (*Task 1* for months 1-18), including: (*Task 1-a*) to test the effects of the compounds on prostate cancer cell proliferation, apoptosis, cell invasion, expression of PSA and other genes (see Figures 1-4 & 9 and Table 1); and (*Task 1-b*) to test the effects of long-term treatment with the compounds on prostate cancer cell growth. In addition to the task, we further screened for newly synthesized DHEA derivatives and identified a compound (PM-IV-7) with not only similar/stronger antiandrogenic activity to/than ADEK, HAD, and OADK, but also marginal estrogenic activity (see Figures 5-8). The effects of this new compound on cell proliferation and the expression of PSA and AR in prostate cancer were additionally investigated (see Figure 9).

Body

Training accomplishments:

I had been away from research laboratory for four years (July 2005 – June 2009) during the pathology training (residency and clinical fellowship). The opportunity to work in the research laboratory (devoting 50% effort for 12 months) is very helpful in updating my research training.

Research accomplishments:

Effects of ADEK, HAD, and OADK on cell proliferation. Using MTT (thiazolyl blue) assay, we first examined androgenic/ antiandrogenic effects of ADEK, HAD, and OADK, in comparison with those of HF, on cell proliferation of six prostate cancer cell lines with different AR status. LNCaP and CWR22Rv1 express a mutant AR T877A and a mutant AR H874Y, respectively. PC-3(AR)2 and PC-3(AR)9 are stable clones of AR-negative PC-3 with wild-type AR under control of a cytomegalovirus promoter [7] and a natural AR promoter [6,8], respectively. The cell lines were cultured for 2 (figure not shown), 4 (figure not shown), or 6 (Figure 1) days in the presence or absence of 1 nM DHT and different concentrations of HF, ADEK, HAD, or OADK. In LNCaP, DHT or HF increased cell growth by nearly 100% after 6-day culture (Figure 1A, lanes 1 *vs.* 2 or 9), whereas ADEK, HAD, and OADK, except 1 μM OADK, showed marginal (<10%) growth induction in the absence of androgens (lanes 1 *vs.* 3-8). ADEK (0.1 and 1 μM), HAD (1 μM), and OADK (0.1 μM), but not HF, significantly antagonized the effect of DHT (lanes 9 *vs.* 10-16). Similarly, in CWR22Rv1, HF, ADEK, HAD, and OADK, except 1 μM OADK, showed marginal (<10%) growth induction in the absence of androgens (Figure 1B, lanes 1 *vs.* 2-8). HF (1 μM), ADEK (0.1 and 1 μM), HAD (1 μM), and OADK (0.1 μM) significantly antagonized the DHT effect (lanes 9 *vs.* 10-16). In PC-3(AR)9, DHT increased cell growth by only 15% (Figure 1D, lanes 1 *vs.* 9). Although HF, ADEK, HAD, and OADK showed marginal (<8%) growth induction in the absence of androgens (lanes 1 *vs.* 2-8), these compounds did not significantly antagonize the effect of DHT (<6%; lanes 9 *vs.* 10-16). In PC-3(AR)2 (Figure 1C), PC-3 (Figure 1E), and DU145 (Figure 1F), DHT, HF, and/or each of the 3 steroid derivatives showed little effects on cell

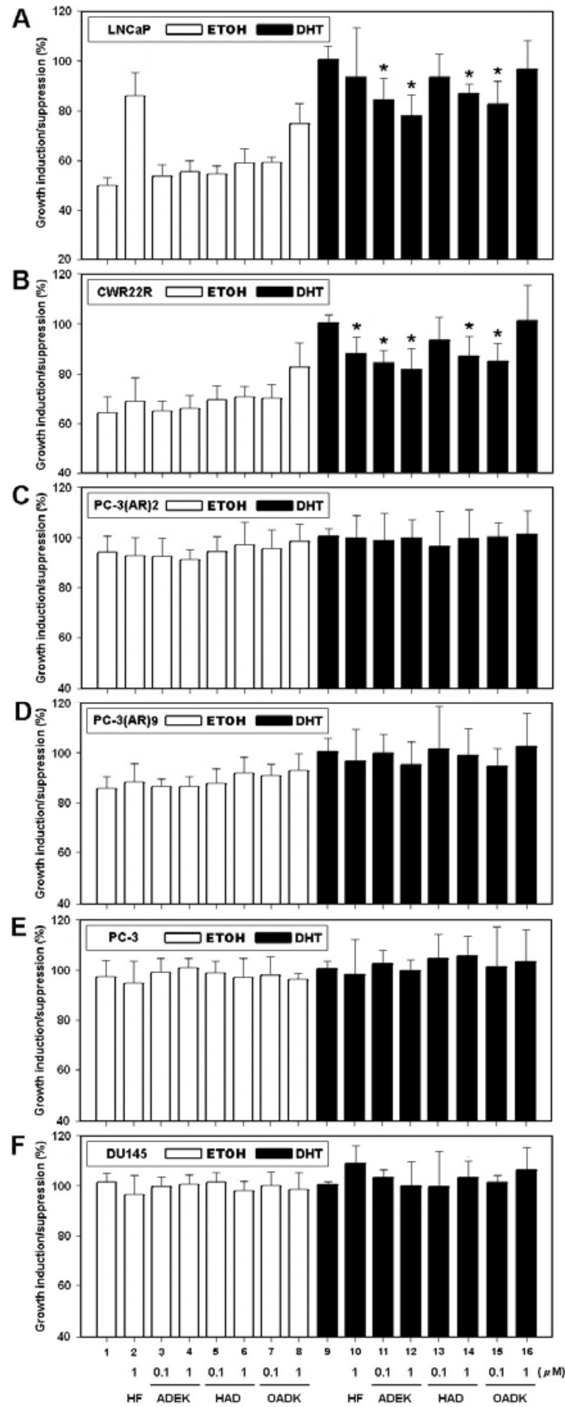


Figure 1. The effects of DHEA derivatives on cell proliferation. LNCaP (A), CWR22Rv1 (B), PC-3(AR)2 (C), PC-3(AR)9 (D), PC-3 (E), or DU145 (F) cells were cultured for 6 days with different concentrations of HF, ADEK, HAD, OADK in the absence [ethanol (ETOH); white bars] or presence (black bars) of 1 nM DHT, as indicated. The MTT assay was performed and growth induction/suppression is presented relative to cell number with DHT treatment in each panel (ninth lanes; set as 100%). Values represent the mean ± SD of at least three determinations. *p<0.05 (*vs.* DHT for lanes 10-16; analyzed by Student's *t*-test).

growth. We also performed MTT assay in the same cell lines with treatment of Adiol (instead of DHT) and the antiandrogenic compounds. However, up to 10 nM of Adiol (physiological concentrations in men are ~5 nM [3]) did not significantly increase the growth of any of the six cell lines, and, therefore, only marginal suppression by steroid derivatives were seen (figure not shown).

Anti-DHT effects of ADEK, HAD, and OADK on apoptosis. Using DNA fragmentation (TUNEL) assay, we next assessed antiandrogenic effects of ADEK, HAD, and OADK on apoptosis. Prostate cancer cell lines were cultured for 3-5 days in the presence of 1 nM DHT and different concentrations of HF, ADEK, HAD, or OADK. Apoptotic indices were determined by fluorescence microscopy. As summarized in Table 1, ADEK (0.1, 1 μM), HAD (1 μM), and OADK (0.1, 1 μM) were found to induce apoptosis in LNCaP and CWR22Rv1 cells.

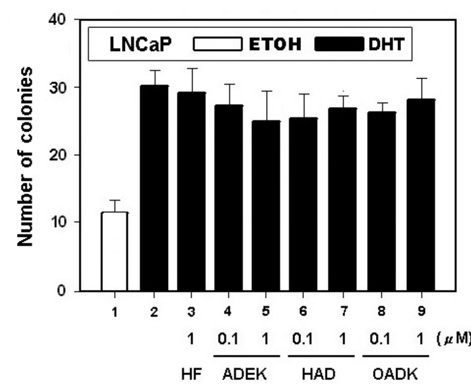
Table 1. Apoptosis in prostate cancer cell lines following the treatment of DHEA derivatives.

	LNCaP		CWR22R		PC-3(AR)2		PC-3(AR)9		PC-3	
Treatment	72h	120h	72h	120h	72h	120h	72h	120h	72h	120h
ETOH	1.2	1.5	2.8	2.9	3.9	4.8	3.6	3.6	1.3	1.4
HF (1 μM)	1.4	2.1	6.1	8.2	4.5	5.8	5.2	5.5	1.1	1.5
ADEK (0.1 μM)	12.4	16.4	12.5	18.6	4.0	5.9	6.5	8.9	2.0	1.9
ADEK (1 μM)	18.5	27.8	14.4	20.1	5.7	5.9	8.0	9.9	1.6	1.7
HAD (0.1 μM)	3.6	5.5	3.9	4.6	4.3	5.1	6.0	8.3	1.5	1.5
HAD (1 μM)	13.8	20.8	13.0	17.7	4.7	5.0	6.9	8.2	1.2	2.0
OADK (0.1 μM)	14.9	21.4	13.3	19.9	6.0	7.3	8.1	10.2	2.2	2.4
OADK (1 μM)	18.9	18.1	15.7	19.5	4.9	6.1	10.3	11.1	1.9	1.8

Apoptotic index = percentage of TUNEL-positive cells in a total of 1,000 cells.

Anti-DHT effects of ADEK, HAD, and OADK on colony formation. We determined the cell survival in a colony formation assay. As shown in Figure 2, the number of colonies formed in LNCaP cells in the presence of DHT was significantly augmented. However, the DHEA derivatives showed marginal to only slight reductions (up to 13%).

Figure 2. The effects of DHEA derivatives on colony-forming. LNCaP cells plated onto the soft agar were cultured for 2 weeks with different concentrations of HF, ADEK, HAD, OADK in the absence [ethanol (ETOH); white bar] or presence (black bars) of 1 nM DHT, as indicated, and stained with methylene blue. Colonies with cell numbers higher than 50 were counted. Values represent the mean ± SD of at least three determinations.



Anti-DHT effects of ADEK, HAD, and OADK on mRNA expression of PSA, AR, and other molecules related to angiogenesis and metastasis. Quantitative reverse transcription (RT)-polymerase chain reaction (PCR) was then performed in AR-positive/PSA-positive prostate cancer cells, LNCaP and CWR22Rv1, in order to assess the antiandrogenic effects of ADEK, HAD, and OADK on cell proliferation (*i.e.* PSA, AR) and angiogenesis/metastasis [*i.e.* basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and matrix metalloproteinase (MMP)-9]. As expected, ADEK (1 μM), HAD (1 μM), and OADK (0.1 μM) suppressed DHT-enhanced PSA expression both in LNCaP and CWR22Rv1 cells (Figure 3A). DHT increased AR mRNA, and only ADEK (in LNCaP) or HAD (in CWR22Rv1) significantly inhibited the DHT-mediated AR expression, although all 3 compounds, as well as HF, showed a tendency to decrease it (Figure 3B). Similarly, DHT could significantly increase the expression of bFGF (Figure 3C), VEGF (Figure 3D), and MMP-9 (Figure 3E) in LNCaP and CWR22Rv1 cells. However, only some treatments significantly antagonized the effects of DHT (ADEK/HAD for VEGF in LNCaP, HAD for VEGF in CWR22Rv1, and ADEK for MMP-9 in CWR22Rv1). Again, 10 nM Adiol did not significantly increase the expression of the molecules, which was only marginally suppressed by the steroid derivatives (figure not shown).

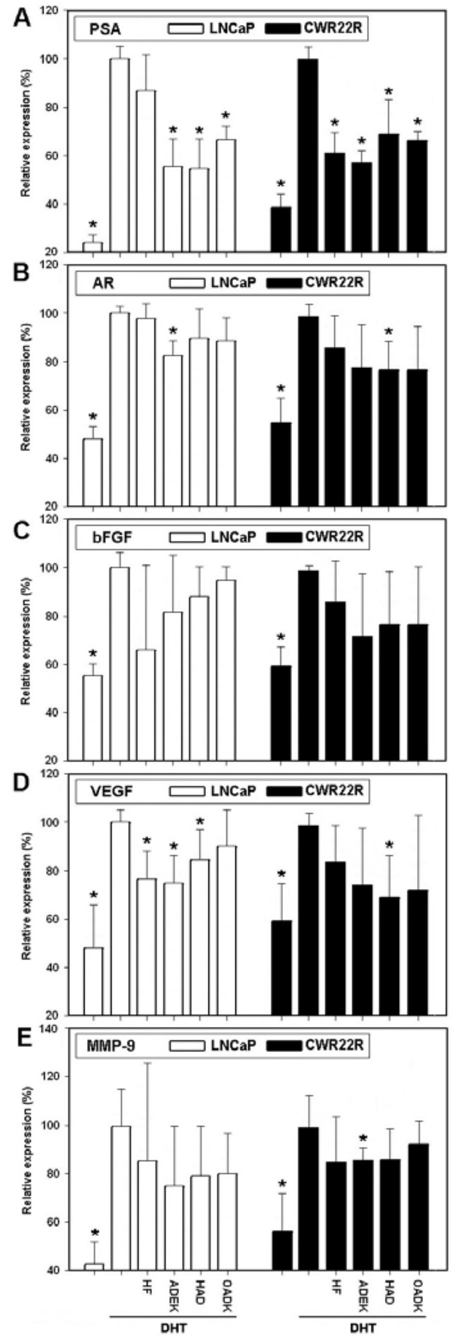


Figure 3. The effects of DHEA derivatives on expression of PSA (A), AR (B), bFGF (C), VEGF (D), and MMP-9 (E). LNCaP (white bars) or CWR22Rv1 (black bars) cells were cultured for 48 h with 1 μM HF, 1 μM ADEK, or 1 μM HAD, or 0.1 μM OADK in the absence or presence of 1 nM DHT, as indicated. Total RNAs from these cells were isolated and reverse transcribed. Real-time PCR was then performed, using each specific primer set. GAPDH was used as an internal control. Expression levels are presented relative to those with DHT treatment in each panel (second lanes; set as 100%). Values represent the mean ± SD of at least three determinations. *p<0.05 (*vs.* DHT; analyzed by Student's *t*-test).

Anti-DHT effects of ADEK, HAD, and OADK on protein expression of PSA. Western blotting analysis was performed, using a PSA antibody (DAKO), as described previously [5,6], in order to determine whether DHEA derivatives inhibit androgen-mediated PSA protein expression in prostate cancer cells. As expected, DHT increased endogenous PSA expression in LNCaP cells over mock treatment (Figure 4, lanes 1 *vs.*

2, please also see Figure 9C). ADEK showed only marginal induction without androgens (lanes 1 *vs.* 3) and antagonized DHT-induced PSA expression (lanes 2 *vs.* 4). In contrast, HAD and OADK showed some agonist effects on PSA expression (lanes 1 *vs.* 5, 7, or 9), although these compounds still showed anti-DHT effects (lanes 2 *vs.* 6, 8, or 10).

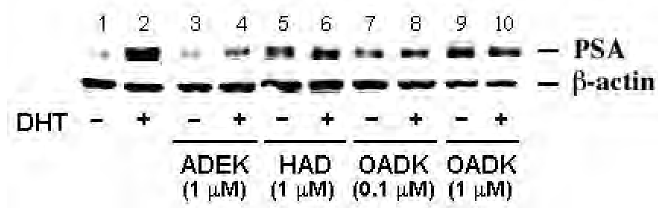


Figure 4. The effects of DHEA derivatives on PSA expression. Cell extracts from LNCaP cultured for 48 h with ADEK (1 μM), or HAD (1 μM), or OADK (0.1 or 1 μM) in the absence or presence of 1 nM DHT, as indicated, were analyzed on Western bots, using an antibody to PSA (upper) or β-actin (lower). The 33 kDa (for PSA) and 43 kDa (for β-actin as an internal control) proteins were detected.

Effects of long-term treatment with ADEK, HAD, and OADK. To determine whether long-term culture with each DHEA derivative as well as a non-steroidal antiandrogen BC leads to any changes in the cells (*e.g.* growth rate, AR expression/mutation, PSA expression, response to androgen supplementation), we have established LNCaP sublines after at least 20-week treatment with ADEK, HAD, OADK, or BC. These sublines are being characterized.

Screening of new DHEA derivatives for their androgenic/antiandrogenic and estrogenic activity. Using a reporter gene assay, we investigated the ability of 10 new steroid derivatives [PM-IV-1 (#1) – PM-IV-10 (#10)] to induce AR/estrogen receptor (ER)-mediated transcriptional activity in the PC-3 prostate cancer cell line. The luciferase (Luc) activity was determined in the cell extracts with transient transfection of a wild-type AR plasmid and an androgen responsive element (ARE)-reporter plasmid (MMTV-Luc) or an ER plasmid and estrogen responsive element (ERE)-Luc. After transfection, the cells were treated with each steroid derivative at 1 μM in the presence or absence of androgen (DHT)/estrogen [17β-estradiol (E2)]. Several of the compounds tested showed marginal (*e.g.* <1.5-fold) induction on AR transcription, as compared with mock treatment (Figure 5A). Most of the

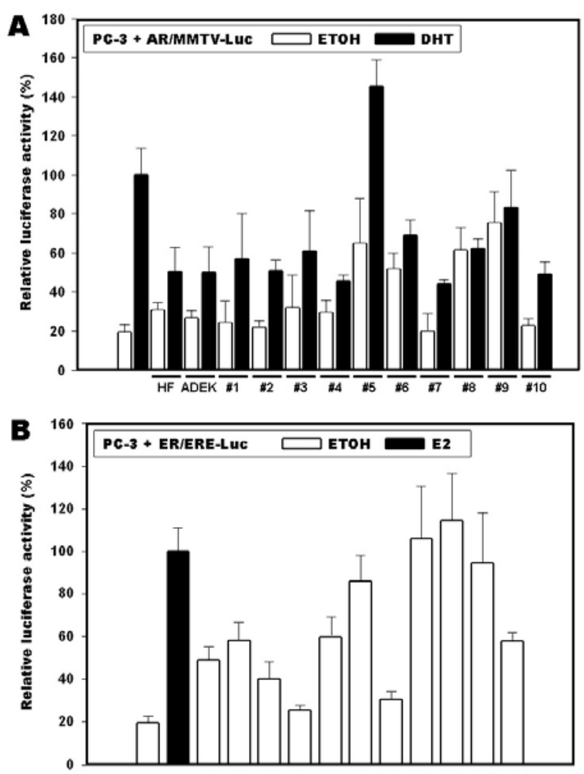


Figure 5. The effects of steroid derivatives on the transcriptional activity of AR (A) and ER (B). PC-3 cells transfected with either pSG5-AR/MMTV-Luc or pSG5-ER/ERE-Luc were cultured for 24 h with 1 μM of various steroid derivatives in the absence (ETOH; white bars) or presence (black bars) of 1 nM DHT or 1 nM E2, as indicated. The Luc activity is presented relative to that in the presence of DHT or E2 (second lanes; set as 100%). Values represent the mean ± SD of at least three determinations.

compounds also showed anti-DHT activity on AR transcription (Figure 5A). However, only a few compounds showed marginal (*e.g.* <1.5-fold) induction on ER transcription, as compared with mock treatment (Figure 5B). In conclusion of our screening, two new steroid derivatives (PM-IV-4 and PM-IV-7) exhibited both marginal androgenic/ estrogenic properties and significant anti-DHT effects. Thus, antiandrogenic effects of PM-IV-4 and PM-IV-7 were further examined in different prostate cancer cell lines and were also compared to those of HF and ADEK.

Anti-DHT effect of PM-IV-4 and PM-IV-7 on AR transcription. Agonist and antagonist effects of PM-IV-4 and PM-IV-7 on AR transcription were assessed in different prostate cancer cell lines [*i.e.* AR-negative PC-3 with transfection of wild-type AR (Figures 6A, 6B, and 6C) or a mutant AR T877A (Figure 6D), LNCaP harboring an endogenous mutant AR T877A (Figure 6E), CWR22Rv1 harboring an endogenous mutant AR H874Y (Figure 6F)], using different ARE-reporter genes [*i.e.* MMTV-Luc (Figures 6A, 6D, 6E, and 6F), synthetic (ARE)4-Luc (Figure 2B), PSA-Luc (Figure 6C)]. Androgenic activity of PM-IV-7 was always marginal (<1.5-fold) in our Luc assays (Figures 6A-6F). In contrast, PM-IV-4 showed partial agonist effects on a mutant AR T877A [3.1- to 5.1-fold over mock treatment (Figures 6D and 6E)], but not (<1.5-fold) on wild-type AR (Figures 6A-6C) or a mutant AR H874Y (Figure 6F). Similarly, in PC-3 with wilt-type AR or a mutant AR T877A, LNCaP, or CWR22Rv1, PM-IV-7 at 1 μM suppressed DHT-induced AR transcription to 51-33%, similar to or

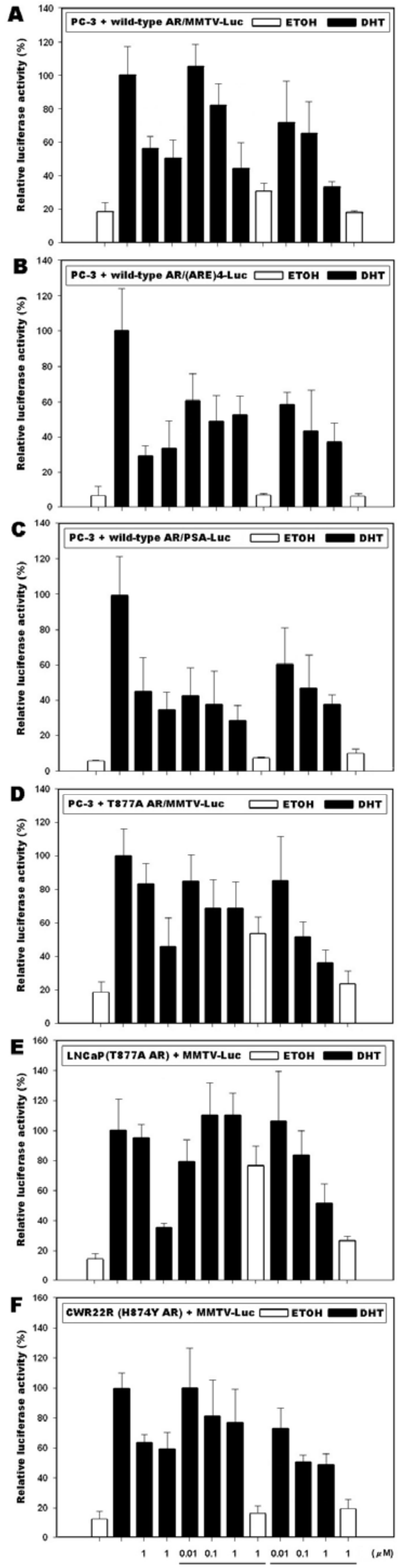


Figure 6. The effects of PM-IV-4 (#4) and PM-IV-7 (#7) on the DHT-induced transcriptional activity of AR. PC-3 (A-D), LNCaP (E), or CWR22Rv1 (F) cells transfected with MMTV-Luc (A, D-F), (ARE)4-Luc (B), or PSA-Luc (C) were cultured for 24 h with 1 μM HF, 1 μM ADEK, or various concentrations of #4 or #7 in the absence (ETOH; white bars) or presence (black bars) of 1 nM DHT, as indicated. In PC-3 cells, an AR expression plasmid [wild-type (A-C) or a mutant T877A (D)] was co-transfected. The Luc activity is presented relative to that in the presence of DHT in each panel (second lanes; set as 100%). Values represent the mean ± SD of at least three determinations.

stronger than the suppression by 1 µM ADEK (Figures 6A-6F). Suppression of DHT-induced wild-type AR transcription by 1 µM PM-IV-4 was similar to or lower than that by ADEK or HF (Figures 6A-6C), whereas PM-IV-4 only marginally inhibited transactivation of mutant ARs (Figure 6D-6F). As reported [2-6], HF functioned as a strong agonist on a mutant (T877A) AR transcription and, therefore, showed no significant anti-DHT (Figures 6D and 6E).

Anti-Adiol effect of PM-IV-4 and PM-IV-7 on AR transcription. We next determined whether PM-IV-4 and PM-IV-7 inhibited Adiol-induced AR transcription by measuring MMTV-Luc activity in PC-3 with wild-type AR (Figure 7A) or a mutant AR T877A (Figure 7B), LNCaP (Figure 7C), or CWR22Rv1 (Figure 7D). Adiol at 10 nM increased AR transcriptional activity to up to 10-fold over mock treatment, and HF totally failed to inhibit the Adiol-induced AR transcription. PM-IV-7 (1 µM) and ADEK (1 µM) repressed Adiol-induced AR transcription to 45-55% and 30-57%, respectively, whereas PM-IV-4 even enhanced it. Because of no anti-Adiol effect of PM-IV-4, in addition to its marginal inhibition of DHT-mediated mutant AR transactivation shown above, only PM-IV-7 was further assessed as to steroid hormone specificity and the effect on prostate cancer cell growth.

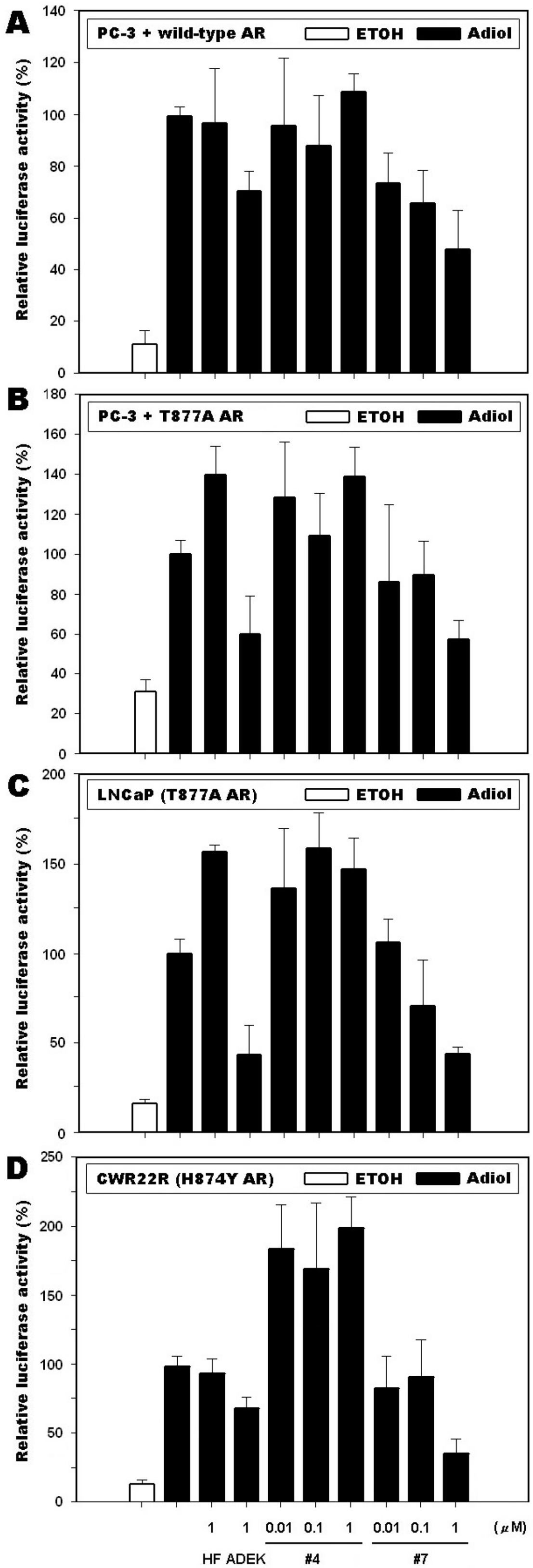


Figure 7. The effects of PM-IV-4 (#4) and PM-IV-7 (#7) on the Adiol-induced transcriptional activity of AR. PC-3 (A, B), LNCaP (C), or CWR22Rv1 (D) cells transfected with MMTV-Luc were cultured for 24 h with 1 µM HF, 1 µM ADEK, or various concentrations of #4 or #7 in the absence (ETOH; white bars) or presence (black bars) of 10 nM Adiol, as indicated. The wild-type AR expression plasmid pSG5-AR was co-transfected in PC-3 cells. The Luc activity is presented relative to that in the presence of Adiol in each panel (second lanes; set as 100%). Values represent the mean ± SD of at least three determinations.

Steroid hormone specificity of PM-IV-7. To assess steroid hormone activity/specificity of PM-IV-7, PC-3 cells were transfected with progesterone receptor (PR)/MMTV-Luc, glucocorticoid receptor (GR)/MMTV-Luc, or ER/ERE-Luc, and treated with or without respective ligand [progesterone (Prog), dexamethasone (Dex), or E2]. Both PM-IV-7 and ADEK showed marginal (<2.0-fold) PR activity (Figure 8A). PM-IV-7 showed partial (up to 30%) suppression on Prog-induced PR transcription, whereas ADEK showed only marginal anti-PR activity (Figure 8A). Similarly, both PM-IV-7 and ADEK showed marginal (<1.2-fold) GR activity (Figure 8B). PM-IV-7 showed partial (up to 55%) suppression on Dex-induced GR transcription, whereas ADEK showed only marginal anti-GR activity (Figure 8B). More importantly, PM-IV-7 possessed only marginal (<1.4-fold) estrogenic activity, similar to that of HF, but, as previously shown [5,6], ADEK partially (2.9-fold, nearly 60% of 10 nM E2 effect) induced ER transcription (Figure 8C). Both PM-IV-7 and ADEK only slightly (up to 16%) suppressed E2-induced ER transcriptional activity (Figure 8C).

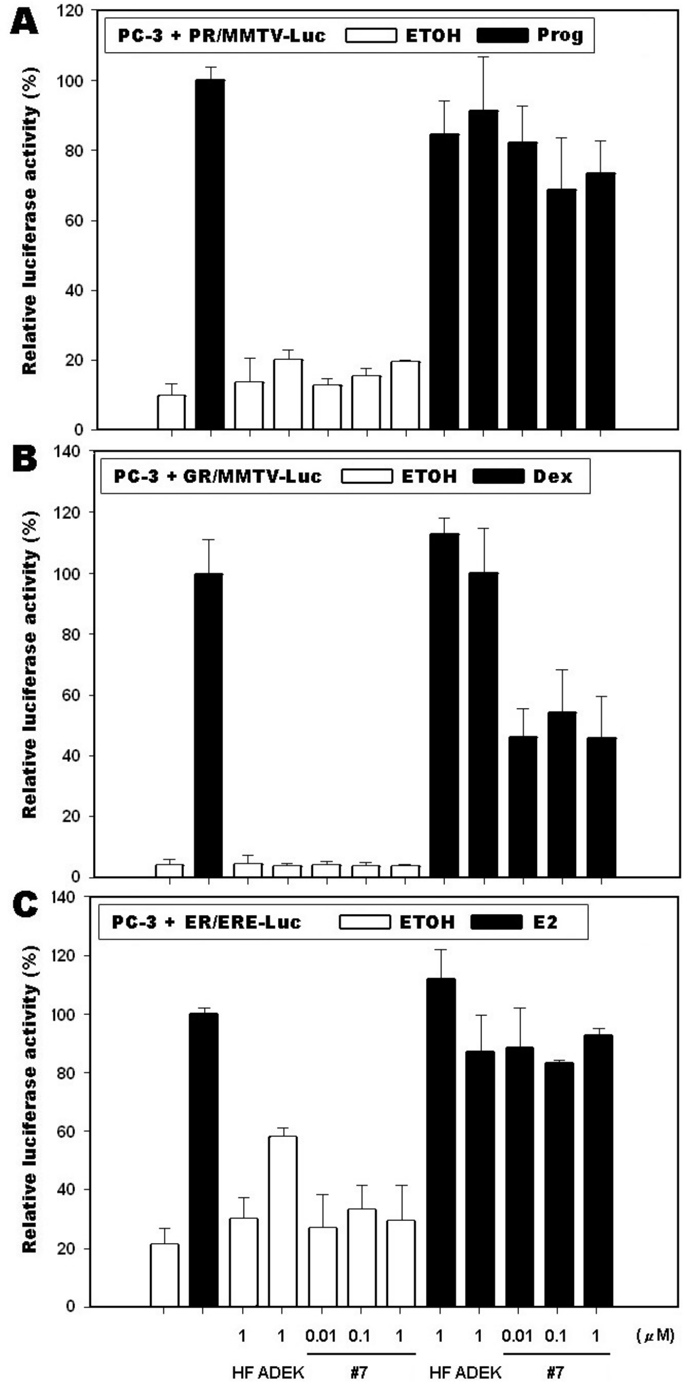


Figure 8. The effects of PM-IV-7 (#7) on the transcriptional activity of PR (A), GR (B), and ER (C). PC-3 cells transfected with steroid receptor and its reporter (PR/MMTV-Luc, GR/MMTV-Luc, or pSG5-ER/ERE-Luc) were cultured for 24 h with 1 μM HF, 1 μM ADEK, or various concentrations of #7 in the absence (ETOH; white bars) or presence (black bars) of ligand (10 nM Prog, 10 nM Dex, or 10 nM E2), as indicated. The Luc activity is presented relative to that in the presence of respective ligand in each panel (second lanes; set as 100%). Values represent the mean ± SD of at least three determinations.

Anti-DHT effect of PM-IV-7 on cell growth. We next compared the effects of PM-IV-7, ADEK, and HF on cell proliferation of AR-positive prostate cancer lines, using MTT assay. As shown in Figure 9A, in LNCaP, DHT increased cell growth by approximately 75% after 4-day culture (lanes 1 *vs.* 2), whereas PM-IV-7 and ADEK showed marginal

(<7%) growth induction in the absence of androgens (lanes 1 *vs.* 5 & 7). Both PM-IV-7 and ADEK significantly antagonized the DHT effect (Figure 9A, lanes 2 *vs.* 6 & 8). Again, HF functioned as an agonist for cell growth of LNCaP. Similarly, as shown in Figure 9B, in CWR22Rv1, DHT increased the growth by approximately 45% after 4-day culture (lanes 1 *vs.* 2), and PM-IV-7, ADEK, and HF showed no significant (<4%) growth induction in the absence of androgens (lanes 1 *vs.* 3, 5, & 7). PM-IV-7 and ADEK, but not HF, significantly suppressed cell growth in the presence of DHT (lanes 2 *vs.* 4, 6, & 8). In an AR-negative PC-3, DHT, HF, ADEK, and PM-IV-7 only marginally affected cell proliferation (figure not shown, also see Figure 1E).

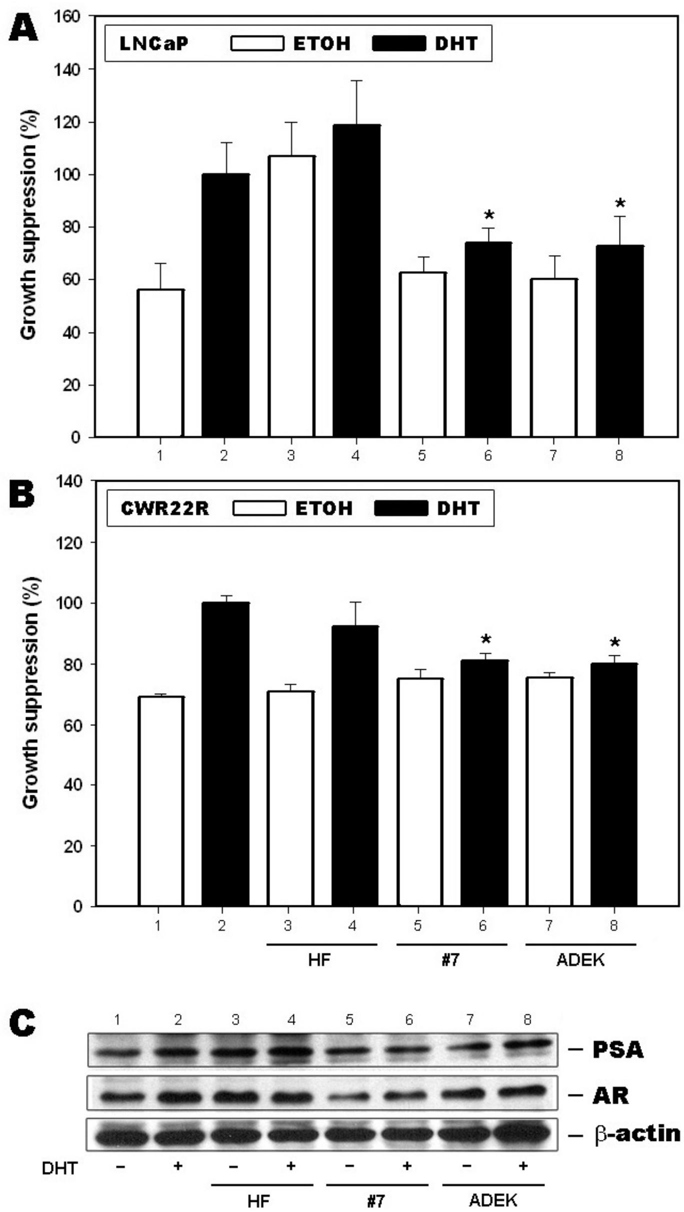


Figure 9. The effects of PM-IV-7 (#7) on cell proliferation and expression of PSA and AR. LNCaP (A) or CWR22Rv1 (B) cells were cultured for 96 h with 1 μM HF, 1 μM ADEK, or 1 μM #7 in the absence (ETOH; white bars) or presence (black bars) of 1 nM DHT, as indicated. The MTT assay was performed and growth induction/suppression is presented relative to cell number with DHT treatment in each panel (second lanes; set as 100%). Values represent the mean ± SD of at least three determinations. *p<0.05 (*vs.* DHT; analyzed by Student's *t*-test). (C) Cell extracts from LNCaP cultured for 48 h with 1 μM HF, 1 μM ADEK, or 1 μM #7 in the absence or presence of 1 nM DHT, as indicated, were analyzed on Western bots, using an antibody to PSA (upper), AR (middle), or β-actin (lower). The 33 kDa (for PSA) and 110 kDa (for AR) proteins were detected. β-Actin expression (43 kDa) was used as an internal control.

Anti-DHT effect of PM-IV-7 on the expression of PSA and AR. Western blotting analysis was performed, using a PSA antibody or an AR antibody (Santa Cruz Biotechnology) in order to determine whether PM-IV-7 inhibits PSA expression in prostate cancer cells. As expected, DHT increased endogenous PSA expression in LNCaP cells over mock treatment (Figure 9C, upper, lanes 1 *vs.* 2, also see Figure 4). Both PM-IV-7 and ADEK showed only marginal induction without androgens (lanes 1 *vs.* 5 & 7) and antagonized DHT-induced PSA expression (lanes 2 *vs.* 6 & 8). Finally, as also shown in Figure 9C (middle), DHT and HF increased endogenous AR expression in LNCaP cells over mock treatment (lanes 1 *vs.* 2 & 3). ADEK had no significant effect on AR expression in the absence of androgens (lanes 1 *vs.* 7), and it antagonized DHT-induced AR expression (lanes 2 *vs.* 8). Interestingly, PM-IV-7 down-regulated AR protein expression in LNCaP both in the absence (lanes 1 *vs.* 5) and presence (lanes 2 *vs.* 6) of androgens via unknown mechanisms.

Key Research Accomplishments

(1) (for *Task 1-a*) Using the prostate cancer cell lines with different AR status, androgenic/antiandrogenic effects of ADEK, HAD, and OADK on cell proliferation/colony formation, apoptosis, and expression of AR, PSA, bFGF, VEGF, and MMP-9 were evaluated, in comparison with HF (Figures 1-4 & 9 and Table 1).

(2) (for *Task 1-b*) LNCaP sublines after long-term treatment (at least 20 weeks) with ADEK, HAD, OADK, or BC were established.

(3) Screening of new DHEA derivatives resulted in identification of a compound that shows marginal androgenic/strong antiandrogenic activity as well as marginal estrogenic activity (Figures 5-9).

Reportable Outcomes

There are no publications from the current project.

Other manuscripts (M1-10) and abstracts (A1-6) during this period include:

(M1) Tavora F, Fajardo DA, Lee TK, Lotan T, Miller JS, **Miyamoto H**, Epstein JI: Small endoscopic biopsies of the ureter and renal pelvis: Pathologic pitfalls. *Am J Surg Pathol* 33: 1540-1546, 2009;

(M2) Duffield AS, Lee TK, **Miyamoto H**, Carter HB, Epstein JI: Radical prostatectomy findings in patients in whom active surveillance of prostate cancer fails. *J Urol* 182: 2274-2279, 2009;

(M3) **Miyamoto H**, Hernandez DJ, Epstein JI: A pathological reassessment of “organ-confined, Gleason score 6” prostatic adenocarcinomas that progress after radical prostatectomy. *Hum Pathol* 40: 1693-1698, 2009;

(M4) **Miyamoto H**, Miller JS, Fajardo DA, Lee TK, Netto GJ, Epstein JI: (Review) Non-invasive papillary urothelial neoplasms: The 2004 WHO/ISUP classification system. *Pathol Int* 60: 1-8, 2010;

(M5) **Miyamoto H**, Sharma RB, Illei PB, Epstein JI: Pitfalls in the use of smoothelin to identify muscularis propria invasion by urothelial carcinoma. *Am J Surg Pathol* 34: 418-422, 2010;

(M6) Lee TK, **Miyamoto H**, Osunkoya AO, Guo CC, Weiss SW, Epstein JI: Smooth muscle neoplasms of the urinary bladder: A clinicopathologic study of 51 cases. *Am J Surg Pathol* 34: 502-509, 2010;

(M7) **Miyamoto H**, Montgomery EA, Epstein JI: Paratesticular fibrous pseudotumor: A morphological and immunohistochemical study of 13 cases. *Am J Surg Pathol* 34: 569-574, 2010;

(M8) **Miyamoto H**, Epstein JI: Transurethral resection specimens of the bladder: Outcome of invasive urothelial cancer involving muscle bundles indeterminate between muscularis mucosae and muscularis propria. *Urology* 76: 600-604, 2010;

(M9) **Miyamoto H**, Brimo F, Schultz L, Ye H, Miller JS, Fajardo DA, Lee TK, Epstein JI, Netto GJ: Low-grade papillary urothelial carcinoma of the urinary bladder: A clinicopathologic analysis of a post-World Health Organization/International Society of Urological Pathology classification cohort from a single academic center. *Arch Pathol Lab Med* 134: 1160-1163, 2010;

(M10) Mir C, Shariat SF, van der Kwast TH, Ashfaq R, Lotan Y, Evans A, Skeldon S, Hanna S, Vajpeyi R, Kuk C, Alkhatee S, Morote J, Van Rhijn B, Bostrom P, Yao J, **Miyamoto H**, Jewett M, Fleshner N, Messing E, Zlotta AR: Loss of androgen receptor expression is not associated with pathological grade, stage, gender nor outcome in bladder cancer: A large multi-institutional study on 472 patients. *BJU Int*, in press;

(A1) Transurethral resection specimens of the bladder: Outcome of invasive urothelial cancer involving muscle bundles indeterminate between muscularis mucosae and muscularis propria. 99th Annual Meeting United States & Canadian Academy of Pathology, March 2010, Washington, DC; *Mod Pathol* 23(Suppl 1): 207A-208A, 2010;

(A2) Identification of Gleason pattern 5 on prostatic needle core biopsy: Frequency of underdiagnosis and morphologic analysis. 99th Annual Meeting United States & Canadian Academy of Pathology, March 2010, Washington, DC; *Mod Pathol* 23(Suppl 1): 190A, 2010;

(A3) Nodular periorchitis: A morphological and immunohistochemical study of 13 cases. 99th Annual Meeting United States & Canadian Academy of Pathology, March 2010, Washington, DC; *Mod Pathol* 23(Suppl 1): 207A, 2010;

(A5) Smooth muscle neoplasms of the urinary bladder: A clinicopathological study of 51 cases 99th Annual Meeting United States & Canadian Academy of Pathology, March 2010, Washington, DC; *Mod Pathol* 23(Suppl 1): 202A, 2010;

(A5) Pitfalls in the use of smoothelin to identify muscularis propria invasion by urothelial carcinoma. 99th Annual Meeting United States & Canadian Academy of Pathology, March 2010, Washington, DC; *Mod Pathol* 23(Suppl 1): 207A, 2010; and

(A6) Bladder cancer invading muscle bundles indeterminate between muscularis mucosa and muscularis propria: A clinocopathologic and immunohistochemical study. 98th Annual Meeting of Japanese Urological Association, April 2010, Morioka, Japan; *Jpn J Urol* 101: 170, 2010.

Conclusion

Using different prostate cancer cell lines, androgenic/antiandrogenic effects of ADEK, HAD, and OADK on cell proliferation, apoptosis, colony formation, and expression of AR, PSA, and angiogenesis/metastasis-related molecules were evaluated. We found that these compounds indeed exhibited antiandrogenic activities, although they were not always significant. Our results thus suggest that ADEK, HAD, and OADK are superior to HF, in terms of androgenic and antiandrogenic properties in prostate cancer cells *in vitro*. We also established LNCaP sublines after long-term treatment with ADEK, HAD, OADK, or BC for further studies. In addition, because of a concern that ADEK, HAD, and OADK possess an estrogenic activity, DHEA derivatives were further screened. One of the new compounds showed the antiandrogenic activity similar to or stronger than that of ADEK, HAD, and OADK, as well as marginal estrogenic activity. We now have four potential compounds, including ADEK, HAD, OADK, and PM-IV-7, for further analyses. We are certain that the remaining of the studies proposed as the *Task 1* will be completed on schedule (by 18th month) and will begin the *Task 2* (from 19th month).

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Appendices

Not applicable.